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EXPRESSION OF CYTOKINES, MATRIX METALLOPROTEASES AND THEIR INHIBITORS IN COLLAGEN-INDUCED ARTHRITIS IN MICE: SALUTARY EFFECTS OF ORAL PROTEASE COCKTAILS

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Aim: The aim of this study is to correlate the expression of selected cytokines with loss of bone and cartilage in a mouse model of rheumatoid arthritis, and to assess the efficacy of protease therapy to alter synthesis of these cytokines.

Methods: After immunization with collagen type II, DBA mice developed inflammatory arthritis by day 28. Three groups of arthritic mice, treated with a commercial protease cocktail, ibuprofen, or saline buffer vehicle, were followed for 2 weeks. At sacrifice (day 42), histologic sections of the rear paws were immunostained by a peroxidase-anti-peroxidase amplification for TNF-α, IL-1 (α and β), IL-1, MMP-1, MMP-3, and a panel of TIMP; loss of cartilage and bone were separately scored, using both histologic and radiographic samples.

Results: Severity of inflammation, and especially of erosion of bone and cartilage, varied from mouse to mouse, and among different joints within a single paw. Increased IL-1 and MMP-3 were closely correlated to cartilagenous erosions (r^2=0.73, 0.65, respectively, both $p < 0.01$). Weaker but significant correlations were observed between these analytes and bony erosion, and between TNF-α, IL-1, and MMP-1 compared with cartilage loss. Expression of TIMPs was variable, and negatively correlated with erosion scores. Protease therapy, and to a significantly lesser extent ibuprofen, markedly reduces the intra-articular expression of TNF-α, IL-1β, IL-6 and MMP-3 (all $p < 0.01$ versus buffer controls), and increases TIMP. Erosive loss of cartilage was reduced by 50-70% in mice treated with proteases.

Conclusion: We conclude that protease therapy strongly diminishes the expression of TNF-α and IL-1β in inflammatory arthritis, and thereby promotes sparing of articular cartilage by favoring expression of TIMPs and diminishing MMPs. Since the erosive phase of osteoarthritis appears similar to that of rheumatoid arthritis, our observations may have ramifications for appreciating the mechanisms and improving the therapy of progressive osteoarthritis.

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DETERMINATION OF CHONDROCYTE VIABILITY WITH FLUORESCENT VITAL STAINING TECHNIQUES

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Aim: The aim of the study was the evaluation of the accuracy of chondrocyte viability determination by using double staining technique with 2'7' bis (2 carboxyethyl 5(6) carboxyfluorescein acetomethyl ester (BCECF) and propidium iodide (P1) and by single staining with acridine orange (AO).

Methods: Primary cultures of human articular cartilage chondrocytes grown on cover slips in Leighton tubes were used. These were stained in culture and after heating to 41-43°C; 44-46°C; 54-56°C and 58-61°C. Prior to staining, the cells were washed with serum free Hank's balanced salt solution. Paired unstained cultures were returned to incubator to determine their growth. Their viability was assessed by trypan blue exclusion, and by cellular growth to confluency.

Results: Cells heated to 41-43°C were over 90% viable, those heated to 44-46°C, 70% viable. Those heated to 54°C and above were non-viable and failed to grow in culture. Bright green fluorescence was observed in BCECF stained cells heated to 54-56°C. Those heated to 58-61°C stained orange red. With AO progressive loss of green fluorescence was noted. With cells heated to 58-61°C having pale green or orange green cytoplasm.

Conclusions: Double staining with BCECF does not differentiate between irreversibly injured and viable chondrocytes cells. Therefore it is a poor indicator of suitability of cartilage for transplantation as used by some investigators. Staining with AO likewise does not differentiate between irreversibly damaged and viable cells. Both techniques produce unequivocal results only with chondrocytes with markedly altered cytoplasm. These results may be important in determining the suitability of stored cartilage for transplantation.

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ORAL ADMINISTRATION OF APOCYNIN IN ZYMOSAN-INDUCED JOINT INFLAMMATION INCREASES RESIDUAL PROTEOGLYCAN SYNTHESIS

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Peroxynitrite formation from O_2 and NO radicals in chondrocytes has been implicated in the IL-1 mediated reduction in proteoglycan (PG) synthesis. Apocynin has been identified as an NADPH-oxidase inhibitor thus preventing 0_2 formation. The aim of this study was to investigate whether orally administered apocynin was able to increase the residual PG synthesis in a model for joint inflammation in mice. 14-week-old C57 black/6 mice were randomly assigned to a control group and a test group (n=7). Test groups were given 3.2, 16 and 80 jig/nil apocynin in the drinking-water, containing 0.05 % ethanol. Control groups received drinking-water water containing 0.05 % ethanol. Supplementation started 4 days prior to zymosan injection and was continued throughout the experiment. At day 4, 6 ml of 30 mg/ml zymosan was injected intra-articularly into the right knee-joint. The left knee-joint served as control. At day 6, mice were sacrificed and the patellae were isolated and analyzed for PG synthesis using $^{35}SO_4$. Injection of zymosan into the knee joint resulted in a decrease in PG synthesis to a level of 33.4% of the control joint 2 days after injection. Supplementation of apocynin 4 days prior to zymosan injection significantly increased the PG synthesis to a level of 44.3% of the control joint at a dose of 80 mg/m apocynin. thus partially preventing loss of PG synthesis capacity (Mann-Whitney U test; $p = 0.011$). No effects of apocynin were observed on the basal proteoglycan synthesis in control joints.
We conclude that apocynin. when administered orally, is able to partially prevent the decrease of PG synthesis of chondrocytes in the patella during acute inflammation. Additionally, these findings suggest that apocynin can be effectively taken up from the GI-tract, processed into its metabolites and reach chondrocytes in the patellae at efficacious concentrations to inhibit NADPH-oxidase. This probably results in a decreased 0_2 production and hence attenuates peroxynitrite in chondrocytes. Additional aspects of joint inflammation and cartilage homeostasis are currently under investigation.